

PATENT
10/001,267
Docket 093/004p

CLAIM AMENDMENTS

1 to 12. *Cancelled*

13. *(Currently amended)* A method for producing differentiated cells from primate pluripotent stem (pPS) cells, comprising :
- a) obtaining a culture of pPS cells;
 - b) optionally initiating differentiation of the pPS cells; and then
 - c) culturing the ~~pPS cells or their progeny~~ cells in a medium containing a histone deacetylase inhibitor, until at least ~60% of the cultured cells have at least three of the following characteristics:
 - antibody-detectable expression of α_1 -antitrypsin (AAT);
 - antibody-detectable expression of albumin;
 - absence of antibody-detectable expression of α -fetoprotein;
 - RT-PCR detectable expression of asialoglycoprotein receptor (ASGR);
 - evidence of glycogen storage;
 - evidence of cytochrome p450 activity;
 - evidence of glucose-6-phosphatase activity; or
 - the morphological features of hepatocytes.
14. *(Previously presented)* The method of claim 13, wherein at least about 60% of the cells have at least five of said characteristics.
15. *(Previously presented)* The method of claim 13, wherein at least about 80% of the cells have at least seven of said characteristics.
16. *(Previously presented)* The method of claim 13, wherein the histone deacetylase inhibitor is *n*-butyrate.
17. *(Previously presented)* The method of claim 13, wherein the histone deacetylase inhibitor is propionic acid, isovaleric acid, or isobutyric acid.
18. *(Previously presented)* The method of claim 13, wherein the histone deacetylase inhibitor is Trichostatin A

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19. *(Currently amended)* The method of claim 13, ~~comprising pre-differentiating the cells wherein~~ differentiation of the pPS cells is initiated by forming embryoid bodies.
20. *(Currently amended)* The method of claim 13, ~~comprising pre-differentiating the cells wherein~~ differentiation of the pPS cells is initiated by culturing in a medium containing dimethyl sulfoxide (DMSO), dimethylacetamide (DMA); hexamethylene bisacetamide, or another polymethylene bisacetamide.
21. *(Previously presented)* The method of claim 13, comprising further culturing the cells in a medium containing a cytokine or hormone selected from glucocorticoids, epidermal growth factor (EGF), insulin, TGF- α , TGF- β , fibroblast growth factor (FGF), hepatocyte growth factor (HGF), IL-1, IL-6, IGF-I, IGF-II, and HBGF-1.
22. *(Previously presented)* The method of claim 21, wherein the cells are cultured in a medium containing at least three of said cytokines or hormones.
23. *(Previously presented)* The method of claim 22, wherein the cells are cultured in a medium containing EGF, TGF- α , and HGF.
24. *(Previously presented)* The method of claim 13, further comprising maintaining the differentiated cells by culturing them in a medium containing a histone deacetylase inhibitor.
25. *(Previously presented)* The method of claim 13, further comprising maintaining the differentiated cells by culturing them in a medium containing n-butyrate.

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26. *(Currently amended)* The method of ~~claim 13~~ claim 27, wherein the pPS cells are human embryonic stem cells.
27. *(Currently amended)* A method for maintaining cells differentiated from an established culture of primate pluripotent stem (pPS) cells, comprising culturing the differentiated cells in a medium containing a histone deacetylase inhibitor, so that at least ~60% of the cultured cells maintain at least three of the following characteristics:
- antibody-detectable expression of α_1 -antitrypsin (AAT);
 - antibody-detectable expression of albumin;
 - absence of antibody-detectable expression of α -fetoprotein;
 - RT-PCR detectable expression of asialoglycoprotein receptor (ASGR);
 - evidence of glycogen storage;
 - evidence of cytochrome p450 activity;
 - evidence of glucose-6-phosphatase activity; or
 - the morphological features of hepatocytes.
28. *(Currently amended)* A method for producing differentiated cells from human embryonic stem (hES) cells, comprising :
- a) obtaining a culture of hES cells;
 - b) optionally initiating differentiation of the hES cells; and then
 - c) culturing the ~~hES cells or their progeny cells~~ in a medium containing a histone deacetylase inhibitor, until at least ~60% of the cultured cells have at least three of the following characteristics:
- antibody-detectable expression of α_1 -antitrypsin (AAT);
 - antibody-detectable expression of albumin;
 - absence of antibody-detectable expression of α -fetoprotein;
 - RT-PCR detectable expression of asialoglycoprotein receptor (ASGR);
 - evidence of glycogen storage;
 - evidence of cytochrome p450 activity;
 - evidence of glucose-6-phosphatase activity; or
 - the morphological features of hepatocytes.

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29. (New) The method of claim 13, wherein the pPS cells are cultured with the histone deacetylase inhibitor without previously initiating differentiation.
30. (New) The method of claim 13, wherein the pPS cells are cultured on an extracellular matrix without feeder cells before contact with the histone deacetylase inhibitor.
31. (New) The method of claim 28, wherein at least about 60% of the cells have at least five of said characteristics.
32. (New) The method of claim 28, wherein at least about 80% of the cells have at least seven of said characteristics.
33. (New) The method of claim 28, wherein the histone deacetylase inhibitor is n-butyrate or Trichostatin A.
34. (New) The method of claim 28, comprising pre-differentiating the cells by culturing in a medium containing dimethyl sulfoxide (DMSO), dimethylacetamide (DMA); hexmethylene bisacetamide, or another polymethylene bisacetamide.
35. (New) The method of claim 28, comprising further culturing the cells in a medium containing at least three cytokines or hormones selected from glucocorticoids, epidermal growth factor (EGF), insulin, TGF- α , TGF- β , fibroblast growth factor (FGF), hepatocyte growth factor (HGF), IL-1, IL-6, IGF-I, IGF-II, and HBGF-1.
36. (New) The method of claim 34, wherein the cells are cultured in a medium containing EGF, TGF- α , and HGF.

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37. (New) The method of claim 27, wherein at least about 60% of the cells have at least five of said characteristics.
38. (New) The method of claim 27, wherein at least about 80% of the cells have at least seven of said characteristics.
39. (New) The method of claim 27, wherein the histone deacetylase inhibitor is n-butyrate.
40. (New) The method of claim 27, wherein the histone deacetylase inhibitor is Trichostatin A
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Upon allowance of the application, please renumber the claims as follows:

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| Claim | 13 | → | 1 |
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| Claim | 28 | → | 16 |
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